

Supporting Information

Identification and Functional Analysis of a Delta Class Glutathione S-transferase

Gene Associated with Insecticide Detoxification in *Bradysia odoriphaga*

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Table S1. Primers used in cloning of *BoGSTd2*, qRT-PCR and RNAi

Primer Name	Sequence (5'-3')	Application	Product Length	Efficiency	R ²
GST-F	GCHGGYGVCAVYWTGAARCC	Conserved region			
GST-R	CCCAWRTCRAAGWABARACG				
Outer	AAGCAGTGGTATCACCGCAGAGT ACGCCGG	5'-RACE			
Inner	AAGCAGTGGTATCACCGCAGAGT				
5-GSP1	CGAAGAATAGACGTTGATTGAC				
5-GSP2	ATCAGATATAACGGCGATGC				
Long	CTAATACGACTCACTATAGGGC AAGCAGTGGTATCACCGCAGAGT	3'-RACE			
Short	CTAATACGACTCACTATAGGGC				
3-GSP1	GAGTATTGAAGCTAAATCCCC				
3-GSP2	CCCTGCAGCGTTGTTCAAATG				
GST2-F	GGGGATTAATTATTAACAAAGAA	Full-length			
GST2-R	TAGAGGGTGCAACGATTCTT				
GST2-ORF-F	<u>CCGG<u>4ATT</u>CATGGGTTGGATT</u> TTACATA	ORF application			
GST2-ORF-R	<u>CCGC<u>TCGAG</u>TTAACCGAAGAAT</u> TTCCT				
GST2-qF	GCCAAGGACGATTCTCTCTATC	qRT-PCR	93 bp	107.5%	0.981
GST2-qR	CCGTTGATATAACGTTCCCAAATC				
18S-qF	CCTGTCTCGGATTAGGAAATG		106 bp	89.6%	0.989
18S-qR	CCCACAGCAACACTTGTAAAG				
Tubulin-qF	CTGGTATGTCGGTGAAGGTATG		95 bp	101.1%	0.983
Tubulin-qR	CCATGCCGACTTCTTCGTAA				
RPS7-qF	GATCCGTTGGTCCGTGAA		104 bp	92.9%	0.951
RPS7-qR	GATTGCGTGTATTCGGTTGG				
RPS15-qF	ATCGTGGCGTCGATTGGAT		164 bp	109%	0.997
RPS15-qR	CTCATTGGTGGGGCTTCCT				
PRL18-qF	CCAACTGGCAAGGGAACCTCT		144 bp	107%	0.979
RPL18-qR	AGCTACGTCTGCGACCTCTA				
T7dsGST2F	<u>TAATACGACTCACTATAGGC</u> GT AAGGAGCGTTG	RNAi			
T7dsGST2R	<u>TAATACGACTCACTATAGGA</u> ATT CCTGAATGCGGC				
T7dsGFPF	<u>TAATACGACTCACTATAGGCC</u> ATG GCCAACACTTGTCA				
T7dsGFPR	<u>TAATACGACTCACTATAGGT</u> TG TCCGAGAACATGTTCC				

Table S2. Overview of information of the sequences in phylogenetic tree

Abbreviations	Species	GeneBank accession number
DmGSTd1	<i>Drosophila melanogaster</i>	AAB26519.1
DaGSTd1	<i>Delia antiqua</i>	ALF04571.1
EbGSTd	<i>Episyrrhus balteatus</i>	CAH58743.1
MdGSTd	<i>Mayetiola destructor</i>	ABG56084.1
CpGSTd	<i>Culex pipiens</i>	AEW07373.1
AgGSTd1-5	<i>Anopheles gambiae</i>	CAB03592.1
AdGSTd1	<i>Anopheles darlingi</i>	ETN67358.1
BoGSTd2	<i>Bradyis aodori phaga</i>	ASK05282.1
LmGSTd	<i>Locusta migratoria</i>	ADR30117.1
CsGSTd3	<i>Chilo suppressalis</i>	AKS40340.1
BmGSTd3	<i>Bombyx mori</i>	NP_001037546.1
CsGSTd2	<i>Chilo suppressalis</i>	AKS40339.1
BmGSTd2	<i>Bombyx mori</i>	NP_001036974.1
TcGSTd2	<i>Tribolium castaneum</i>	AFM57703.1
AfGSTe2	<i>Anopheles funestus</i>	AHC31032.1
AcGSTe2	<i>Anopheles cracens</i>	ACY95463.1
AgGSTe1	<i>Anopheles gambiae</i>	AAL59658.1
SeGSTe	<i>Spodoptera exigua</i>	AHB18378.1
DmGSTe7	<i>Drosophila melanogaster</i>	NP_611329.1
CsGSTe2	<i>Chilo suppressalis</i>	AKS40343.1
BmGSTe1	<i>Bombyx mori</i>	NP_001037197.1
BmGSTu1	<i>Bombyx mori</i>	NP_001108462.1
CpGSTu	<i>Culex pipiens</i>	ARM39010.1
HvGSTu1	<i>Heortia vitessoides</i>	AWX68896.1
CsGSTu	<i>Chilo suppressalis</i>	AKS40352.1
TmGSTz	<i>Tenebrio molitor</i>	AIL23553.1
LmGSTz	<i>Locusta migratoria</i>	AHC08063.1
CsGSTz1	<i>Chilo suppressalis</i>	AKS40351.1
NIGSTz1	<i>Nilaparvata lugens</i>	AFJ75820.1
PrGSTz2	<i>Pieris rapae</i>	APW77583.1
BmGSTz	<i>Bombyx mori</i>	ABF51419.1
BmGSTo3	<i>Bombyx mori</i>	NP_001040435.1
DmGSTo3	<i>Drosophila melanogaster</i>	NP_648234.1
BmGSTo2	<i>Bombyx mori</i>	NP_001037406.1
TmGSTo	<i>Tenebrio molitor</i>	AIL23546.1
LmGSTo	<i>Locusta migratoria</i>	AHC08061.1
AgGSTo1	<i>Anopheles gambiae</i>	AAP13482.1
DmGSTo1	<i>Drosophila melanogaster</i>	NP_648237.1
DaGSTo1	<i>Delia antiqua</i>	ALF04577.1
DmGSTt1	<i>Drosophila melanogaster</i>	NP_610509.2
BmGSTt1	<i>Bombyx mori</i>	NP_001108463.1
LmGSTt1	<i>Locusta migratoria</i>	AEB91980.1
AdGSTt	<i>Anopheles darlingi</i>	ETN68079.1
TmGSTt	<i>Tenebrio molitor</i>	AIL23552.1
DmGSTt4	<i>Drosophila melanogaster</i>	NP_572886.2
TmGSTs	<i>Tenebrio molitor</i>	AIL23550.1
DnGSTs1	<i>Drosophila nasuta</i>	BAR89482.1
BgGSTs	<i>Blattella germanica</i>	AEV23881.1
PpGSTs1	<i>Papilio polytes</i>	BAM19280.1
CsGSTs	<i>Chilo suppressalis</i>	ADD14027.1
BmGSTs	<i>Bombyx mori</i>	BAD91107.1
ApGSTs	<i>Antheraea pernyi</i>	ADC32118.1

Figure legends

Figure S1. cDNA and deduced amino acid sequence of BoGSTd2 from *B. odoriphaga*. The initiation codon (ATG) and termination codon (TAA) are boxed in black. The α -helix and β -pleated sheet are indicated by solid and arrowed lines, respectively.

Figure S2. Three-dimensional structure of BoGSTd2 from *B. odoriphaga*. Three-dimensional model of BoGSTd2 was generated based on the deduced protein sequences by the SWISS-MODEL program using the structure of GSTd1 of *Drosophila melanogaster* as the templates.

Figure S3. Expression and purification of recombinant BoGSTd2. All proteins were expressed in *E. coli* BL21(DE3) using the vector pET28a (+) and purified by Ni-NTA Resin. Lane 1: Molecular weight markers. Lane 2: supernatant of pET28a (+) (no insert) without induction. Lane 3: supernatant of pET28a (+) (no insert) induction with 0.4 mM IPTG. Lane 4: supernatant of recombinant BoGSTd2 without induction. Lane 5: supernatant of recombinant BoGSTd2 after induction with 0.4 mM IPTG. Lane 6: purified recombinant BoGSTd2.

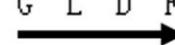
Figure S4. Enzymatic properties of recombinant BoGSTd2. (A) Relative activity of purified recombinant BoGSTd2 at various pH values. (B) Relative activity of purified recombinant BoGSTd2 at different temperatures. (C) Dose-response curves for the inhibition of CDNB conjugating activity of BoGSTd2 by GTx (S-hexylglutathione).

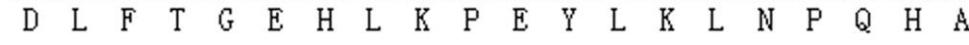
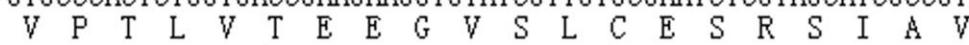
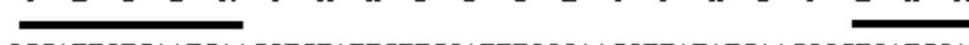
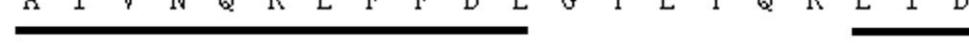
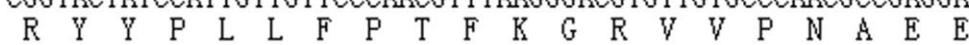
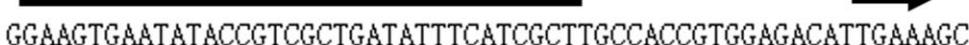
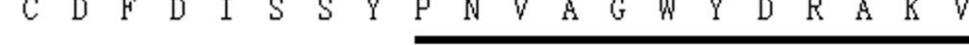
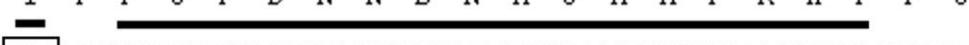
Figure S5. The kinetic properties of recombinant BoGSTd2. (A) Activity of recombinant BoGSTd2 with different concentrations of CDNB. (B) Lineweaver–Burk plot of kinetic properties of purified recombinant BoGSTd2 using CDNB as substrates. (C) Activity of recombinant BoGSTd2 with different concentrations of CHP (Cumene hydroperoxide). (D) Lineweaver–Burk plot of kinetic properties of purified recombinant BoGSTd2 using CHP as substrates. (E) Activity of recombinant BoGSTd2 with different concentrations of H₂O₂. (F) Lineweaver–Burk plot of kinetic properties of purified recombinant BoGSTd2 using H₂O₂ as substrates.

Figure S6. Dose-response curves for the inhibition of CDNB conjugating activity of BoGSTd2 by insecticides. (A) lambda-cyhalothrin; (B) chlorpyrifos; (C) clothianidin.

The data and error bars represent the means and standard error of three biological replicates.

Figure S7. Chromatograms reports of insecticides metabolic assays. (A and B) lambda-cyhalothrin; (C and D) chlorpyrifos; (E and F) clothianidin. The elution time of lambda-cyhalothrin is 3.5 min. The elution time of chlorpyrifos and clothianidin is 1.5 min. Peak areas of residual insecticides were marked in the figure. (A, C and E) Incubation of insecticide and GSH with 20 µg inactive recombinant BoGSTd2. (B, D and F) Incubation of insecticide and GSH with 20 µg active recombinant BoGSTd2.

1 ATTAAGCAGTGGTATCAACCGCAGAGTACGCCGGGGGGGGGATTAAATTATAACAAA
 60 GAAAAAAAATCTCACAAAAAATGGGTTGGATTTCACATATTGGACGGATCTGCTCCC
 M G L D F Y I L D G S A P

 120 TGCAGCGTTGTTCAAATGACTGCGAAGGCTGTTGGCGTTGAATTGAATTAAAACCGGTT
 C S V V Q M T A K A V G V E L N L K P V

 180 GATCTGTTCACTGGTGAACATCTGAAACCAGAGTATTGAAGCTAAATCCCCAGCATGCC
 D L F T G E H L K P E Y L K L N P Q H A

 240 GTGCCCACTCTGGTGACCGAACAGGTGTATCGTGTGCGAATCTCGTAGCATGCCGTA
 V P T L V T E E G V S L C E S R S I A V

 300 TATCTGATCGAAAGATATGCCAAGGACGATTCTCTCTATCCCAAGGATCCATTAAAACGG
 Y L I E R Y A K D D S L Y P K D P L K R

 360 GCCATTGTCAATCAACGTCTATTCTCGATTGGAACGTTATATCAACGGCTGATCGAT
 A I V N Q R L F F D L G T L Y Q R L I D

 420 CGGTACTATCCATTGTTGTTCCCAACGTTAACGGACGTGTTGTGCCAACGCCAGGAA
 R Y Y P L L F P T F K G R V V P N A E E

 480 CAATTGAAAGAACGCTGTCGGCTTTTGAAACACTTTCTGGATGGAAATGCATTGCTGCT
 Q L K E A V G F L N T F L D G N A F V A

 540 GGAAGTGAATATAACCGTCGCTGATATTCACTCGCTGCCAACCGTGGAGACATTGAAAGCT
 G S E Y T V A D I S S L A T V E T L K A

 600 TGCGACTTCGATAATCAGTTCGTATCCGAATGTTGCCGGTTGGTATGACCGTGCCAAAGTC
 C D F D I S S Y P N V A G W Y D R A K V

 660 ATCACTCCAGGATTGATAACAACGAGAACCATGGGGCCGCATTCAAGGAAATTCTCGGT
 I T P G F D N N E N H G A A F R K F F G

 720 TAAACTGGATGTGCCAGTTAGGCCTCAGAGTCAGAAATTATCAAACGAATAATGAATG
 780 TAAATGCACTGCAGAGCATCATGTTGTCATTGGTGAACAAATAATTGATTAAGAATC
 840 GTTGCACCTCTAAAAAAAAAGTACTCTGCCTGATACCACTGCTTGCCTA
 900 TAGTGAGTCGTATTAG

Figure S1

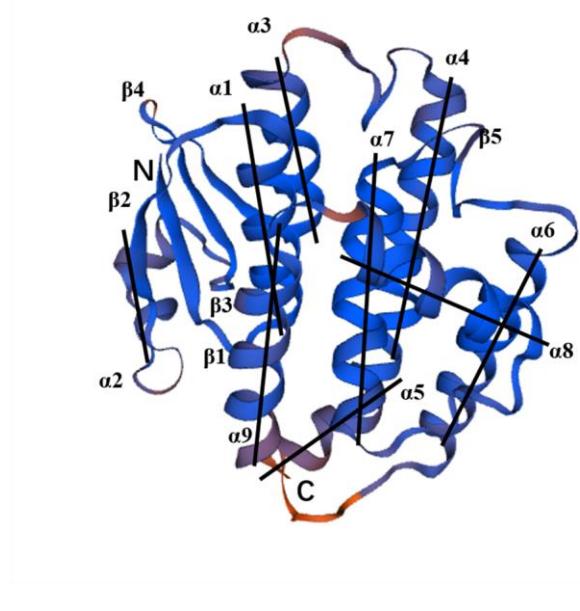


Figure S2

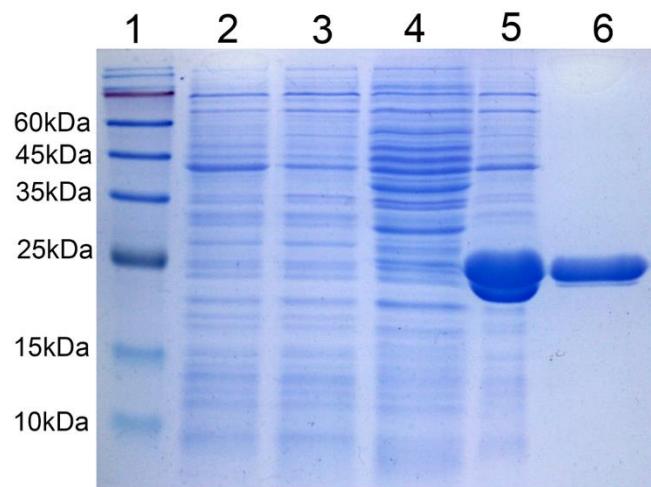


Figure S3|

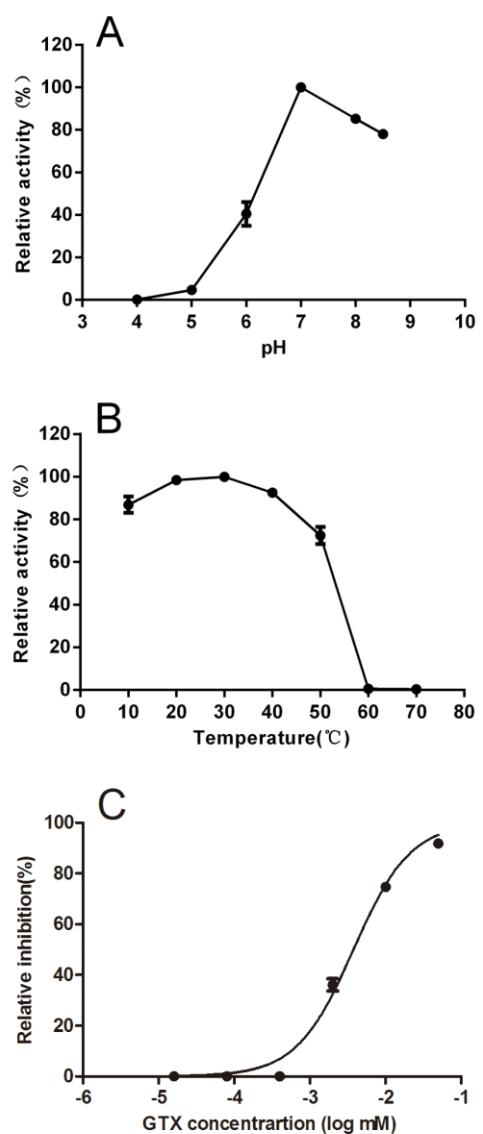


Figure S4

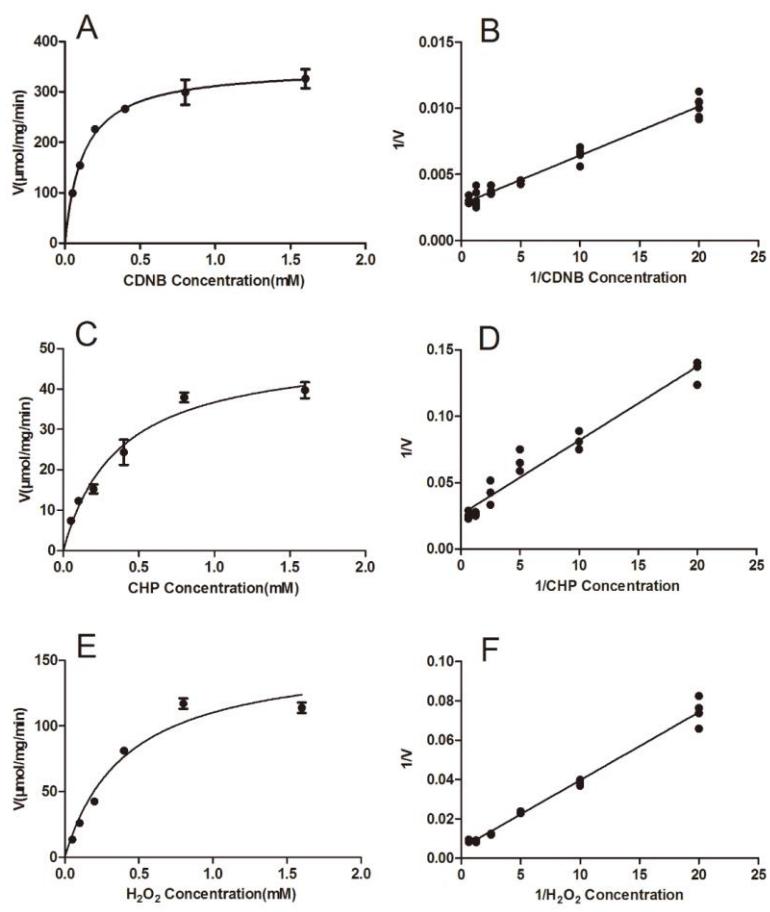


Figure S5

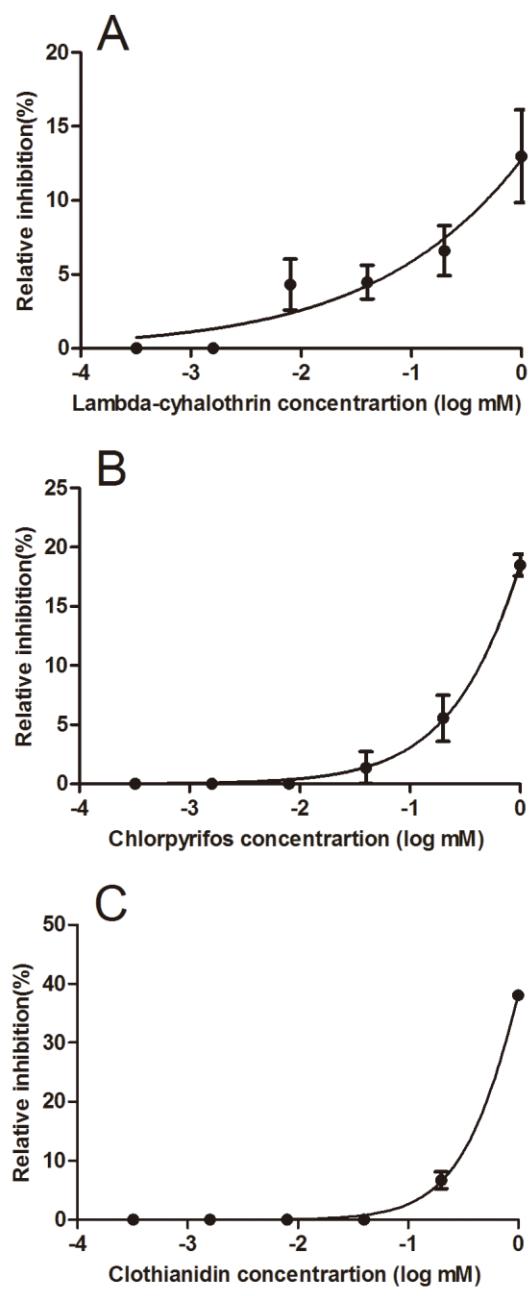


Figure S6

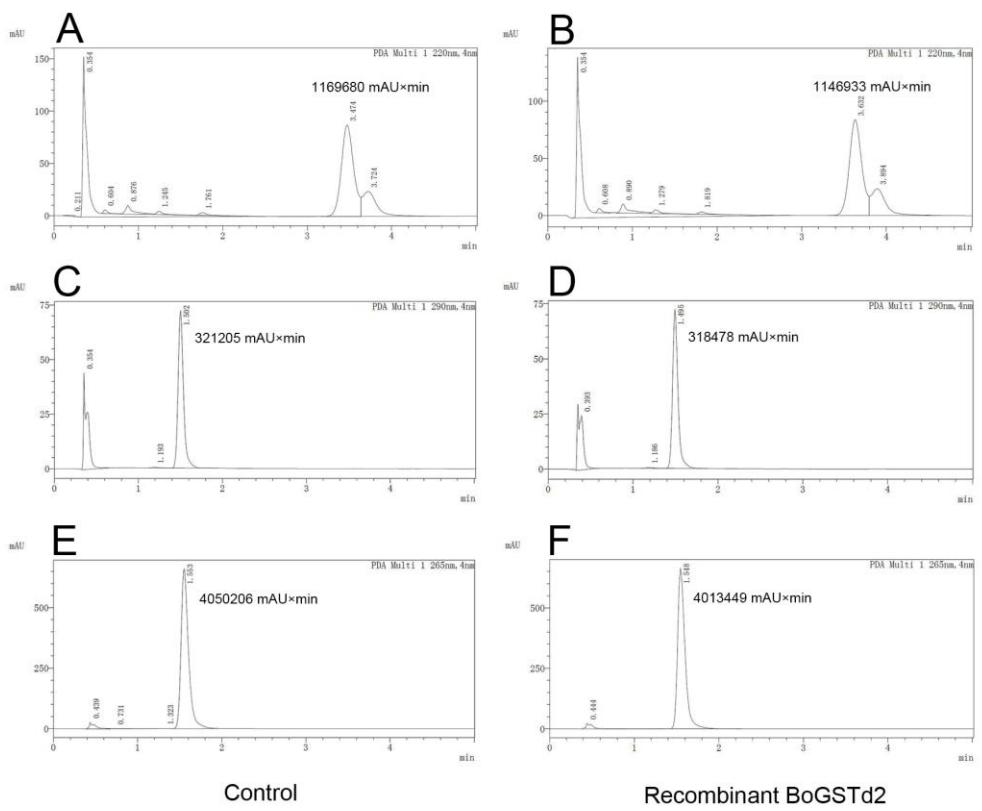


Figure S7